DIFFERENTIATION OF SPOROCARPS IN AZOLLA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 105

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(WITH PLATES XXXI AND XXXII)

A summary of the work on Azolla up to 1844 may be found in GRIFFITH'S (1) account of Azolla and Salvinia. Working on *Azolla pinnata*, GRIFFITH began his account with the development of the sporocarps. The earliest stage described showed two sporocarps within a hoodlike involucre. Each sporocarp consisted of a cup-shaped integument and a cellular body, the "nucleus,"[1] which was surrounded by the "integument"[2] and projected from it. Later the "nucleus" elongated and the "integument" grew out so rapidly as finally to inclose it. At a still later stage, simple capitate "filaments"[3] of cells developed about the base of the "nucleus." Up to this point, the development in the two organs had been similar. After this, there might be a development of either the "nucleus" or the cellular projections about its base, but in no case did both continue growth. In cases where the further growth was in the "nucleus," GRIFFITH describes it as developing into the "so-called male organ" of former writers. This organ consisted of an egg-shaped body, bearing a calyptra and containing a large yellow sac capped by a peculiarly stratified body. The first change in the development here was a granular condensation of the materials of the "nucleus." When, on the other hand, only the "so-called female organs" developed, the first step was the appearance of a granular mass in the swollen tip of each of the "filaments," which had appeared in centrifugal order about the base of the "nucleus."

Nearly thirty years later (1873), STRASBURGER (2) wrote *Ueber Azolla*, in which he gave a full account of the mature sporocarps, but said little about their development. He found in the early stages of

[1] From GRIFFITH'S figures it is evident that this "nucleus" is the megasporangium.

[2] The "integument" is the sporocarp wall.

[3] These "filaments" are evidently the young microsporangia, which arise from the massive stalk of the megasporangium.

the megasporocarp a single sporangium developing, and when later he found only one megaspore in the mature sporocarp he naturally inferred that only one sporangium had appeared. Referring to GRIFFITH'S work, STRASBURGER suggested that in case more than one sporangium did appear in a sporocarp, it is more likely that all sporangia should have appeared simultaneously, and that later those about the base of the large central sporangium should have aborted. In the microsporocarp he found microsporangia, in numbers as high as forty, arising from a short "columella." He further said that he found no sign of the club-shaped ending of the columella which MEYEN had described earlier.

In 1889 STRASBURGER (3) reinvestigated the subject. According to that account, the primordia of both male and female sori are alike, since in each case there arose at first a short-stalked "macrosporangium" at the apex of the columella. In the "macrosporocarp" this went on developing, but in the microsporocarp its growth was soon inhibited, while from the columella beneath it microsporangial primordia were constantly arising. He suggested that the development of one or the other might depend upon the method of nutrition.

In 1893 CAMPBELL (4), referring to *Azolla filiculoides*, wrote:

> For some time each sporocarp rudiment grows by a three-sided apical cell. Next a slight outgrowth is observed near the base of the young sorus, which forms a ring-shaped projection; this is the beginning of the indusium or sporocarp wall. . . . . From this point, the two sorts of sori differ. In the macrosporic one, the apical cell forms at once the body of a single sporangium; in the microsporic it forms a columella, from which latter the microsporangia appear as lateral outgrowths.

In speaking of the megasporocarp he said:

> When the sporangium is about half grown, the outer cells of the very short stalk grow out into short papillae, which apparently are abortive sporangia, as they show divisions which recall the earlier ones in the macrosporangium. Their position corresponds to that of the microsporangia, so that although formed much later than the macrosporangium, it is pretty safe to assume that Azolla is derived from some form in which, as in Salvinia, there are several macrosporangia in the sporocarp. . . . . In the male sorus, as we have already seen, the apical cell of the young rudiment does not form a sporangium, but gives rise to a central columella or placenta from which microsporangia arise laterally, while the end projects as a cylindrical body. This latter was observed by MEYEN, but STRASBURGER seems for some reason to have overlooked it. I found it in all of my sections of the male sorus.

In 1898 GOEBEL (**5**) again investigated the method of sporocarp development in *A. filiculoides*. He accepted STRASBURGER'S later account of the development of the microsporocarp, but in the megasporocarp he found in the later stages of development primordia of microsporangia which had aborted.

In CAMPBELL'S revised edition of *Mosses and ferns* (**6**, 1905), he refers to GOEBEL'S account and states that "in the microsporangial sorus the apex of the receptacle, which probably represents an abortive macrosporangium, forms a columella, from whose base the microsporangia develop." He uses the illustration of the microsporocarp which he had used in the original paper, and shows the columella as a club-shaped body which has little resemblance to a sporangium.

INVESTIGATION

Working on *Azolla caroliniana*, I have traced the development of the sporocarp, paying particular attention to the stage at which microsporocarp and megasporocarp are definitely differentiated.

The youngest stage in which the sporocarp can be readily recognized shows a young sporangium consisting merely of an apical cell and a short stalk, about whose base there is a ringlike projection (*fig. 1*). This sporangium, which holds the central position in the mature sporocarp, is the megasporangium, and is of the ordinary leptosporangiate type.

The projecting ring of cells about the base of the sporangium, by subsequent growth outward and upward, forms the wall of the sporocarp. Growth of the sporangium and growth of the sporocarp wall go on simultaneously, so that by the time the primary tapetal cells are cut off in the capsule of the sporangium, the two-layered sporocarp wall stands almost as high as the sporangium (*fig. 3*). While the development of the capsule of the sporangium has gone on in the regular leptosporangiate way, there has been no such elongation of the pedicel as might be expected. The divisions of the cells of the pedicel, for the most part, have been anticlinal rather than periclinal to the apical cell, so that the stalk is massive, almost as great in diameter as is the capsule (*fig. 3*). Growth in the capsule now continues in the usual way until there is finally a sporangium having one layer of wall cells

and a single layer of tapetal cells surrounding a mass of eight megaspore mother cells in synapsis. There is no trace of an annulus of any sort to be found either at this or any later stage. By this time the sporocarp wall has usually grown up so as almost to inclose the sporangium, while the stalk of the sporangium is likely to be at least four or five cells in diameter (*fig. 4*). While the mother cells are in synapsis, still forming a compact tissue surrounded by a layer of tapetal cells, some of the outer cells of the sporangium stalk enlarge so as to form short papillae (*fig. 5*). These enlarged cells have nuclei larger than those of the other stalk cells, and very soon it becomes evident that each of them is the apical cell of a young sporangium (*fig. 6, mc*). This is the first appearance of microsporangia in the sporocarp. Shortly after the microsporangia appear, the walls of the tapetum in the megasporangium disappear, leaving tapetal nuclei free in a mass of cytoplasm. Surrounded by this tapetal cytoplasm and nuclei, the megaspore mother cells have now separated from each other and become rounded off (*fig. 6*).

In a few cases the growth of the megasporangium is inhibited at this point. When this occurs the megaspore mother cells become shrunken, the tapetal nuclei appear small and more densely granular than in the healthy sporangia, and the cytoplasm takes on a coarse, loosely granular appearance. As this breaking down goes on in the megasporangium, more and more microsporangial primordia appear at its base. This, however, is not the usual course of events. Ordinarily the growth of the megasporangium is not inhibited until after it has passed the spore mother-cell stage.

In almost every case, the megasporangium continues its growth in size while the young microsporangia are developing at its base. Finally the megaspore mother cells divide simultaneously (*fig. 7*) to produce the eight tetrads (*fig. 8*). Even while this division of the megaspore mother cells is taking place, growth in the microsporangia continues, as is readily seen by the fact that cell division sometimes takes place there (*fig. 7, mc*). It is therefore after the formation of the megaspore tetrads (*fig. 8*) that the critical stage in the development is reached.

At this stage the sporocarp wall, which consists of only two layers of cells, has grown up beyond the apex of the megasporangium and

has become almost if not completely closed by the greater elongation of the cells of the outer layer. The general shape of the sporocarp is oval, flattened on one side, and with a beak of enlarged cells at the top. Just inside this wall one often finds unattached filaments of Anabaena lying in the space above the megasporangium. At the base of the sporocarp there arises a massive stalk bearing sporangia. Of these sporangia, the oldest, which holds the central position, is the megasporangium, while the very young microsporangia form one or more whorls about its base. Within the megasporangium wall, of a single layer of cells, is found a mass of cytoplasm in which a large number of small nuclei, of tapetal origin, are lying free, and in the center, surrounded by the most densely granular of this cytoplasm, eight tetrads of spores are lying free from each other.

The question now is whether the megasporangium or the group of microsporangia, all of which are apparently vigorous, shall go on developing. If the megasporangium develops, there will be a megasporocarp; while if the microsporangia develop, there will be a microsporocarp.

In the megasporocarp, thirty-one of the megaspores abort, while one, which usually holds the central position in the sporangium, continues growth (*fig. 9*). The megasporangium increases in size, but there is no further elongation of the stalk, and the young microsporangia at its base cease their growth. As the megasporangium increases in size, it finally completely fills the sporocarp, and the young microsporangia are squeezed down against the stalk until they are hardly recognizable. An abnormal condition, which I found in two megasporocarps, shows two of the megaspores developing, while only thirty abort.

In the microsporocarp, the abortion of the spores in the megasporangium goes a step farther than in the megasporocarp, and we find all thirty-two megaspores aborting. Within the megasporangium the cytoplasm takes on a coarsely granular appearance, the tapetal nuclei are dense shrunken masses, and the megaspores, which may hang together in tetrads or may fall apart as separate spores, are shrunken and shapeless (*fig. 10*). As this abortion goes on, the stalk of the megasporangium elongates somewhat and new microsporangial primordia continue to appear below the older ones, which are

increasing rapidly in size. The microsporangia arise in basipetal succession, have long slender stalks, and each sporangium develops sixteen spore mother cells. As the growth of the stalks in length and of the capsules in size continues, there is a consequent broadening of the sporocarp, which makes of the mature microsporocarp a round body, quite different from the more elongated megasporocarp. By the time the oldest of the microsporangia have reached the mother-cell stage, the megasporangium has usually collapsed, so that unless one happens to get a median section of the sporocarp (*figs. 11, 11a*) it is likely to escape notice. However, I found several microsporocarps in which this collapse of the megasporangium did not occur. In these cases the cytoplasmic contents of the megasporangium had broken down and had to a large extent been resorbed, but the sporangium wall remained turgid, while the cells of the stalk at the base of the sporangium had grown up into the cavity left by the breaking-down of the cytoplasm (*figs. 12, 13*). In one case (*fig. 13*) this growth had continued until the sporangium was nearly filled with vegetative tissue.

DISCUSSION

While GRIFFITH, in 1844, interpreted the megasporangium as a male organ and the microsporangia as female organs, as R. BROWN and MEYEN had before him, he gave an accurate account of the order in which the various structures of the sporocarp appear. Since the appearance of STRASBURGER'S *Ueber Azolla*, writers seem largely to have overlooked GRIFFITH'S work. Doubtless this is due to the fact that STRASBURGER, who worked largely on the very early and the mature stages of the development, without catching the intermediate stages, suggested that probably GRIFFITH'S account would not hold true, since it seemed more probable that the sporangia would appear simultaneously in the megasporocarp as they did in the microsporocarp. It is interesting to note that sixteen years after *Ueber Azolla* appeared, STRASBURGER again gave an account of Azolla, in which he affirms that the primordia of the two sporocarps are alike, since each begins with the development of a megasporangium, which in the microsporocarp soon aborts. It is evident that he no longer thought that the development of sporangia in the microsporocarp is simultaneous, and so his former inference, that probably the mega-

sporocarp is like the microsporocarp in this particular, no longer has any weight.

CAMPBELL, writing four years after the appearance of STRASBURGER'S second paper, has evidently not given close attention to this later account of the development, or he might have been led to make a more careful study of the columella, which he found in all his preparations of the microsporocarp. That this columella actually represents an abortive megasporangium seems not to have occurred to him, although STRASBURGER'S paper would have suggested it. The nearest approach that I found to such a columella as CAMPBELL figures was in the few cases where the vegetative cells of the stalk had grown up into the cavity left by the collapse of the cytoplasmic contents of the megasporangium (*figs. 12, 13*). Any section, except the median, of such a sporangium as that shown in *fig. 13*, would show merely a club-shaped mass of cells; but the median section shows very clearly the remains of the broken-down cytoplasm and nuclei.

To one who has noted the megasporangium beginning to develop in the microsporocarp and then aborting, the interpretation of the sporangial primordia about the base of the megasporangium in the megasporocarp as microsporangia is natural. CAMPBELL, having missed this first point, seems to have missed the second one also, since he infers that the sporangial primordia in the megasporocarp are megasporangial, and so to be compared with Salvinia. He overlooks the fact that in Salvinia the appearance of sporangia is much more nearly simultaneous than in Azolla.

Of all the accounts of Azolla, GOEBEL'S most nearly portrays the course of events as I saw it. Yet even he accepts STRASBURGER'S statement that the abortion of the megasporangium in the microsporocarp occurs early. While at first sight this would seem to be the natural course of events, abortion after the formation of the megaspores really carries the reduction in the megasporangium only one step farther than in the megasporocarp. Long ago STRASBURGER saw cases in the megasporocarp where two spores developed while thirty aborted. This condition, which I have seen a few times, was readily looked upon as a reversion to the condition in which more than one spore developed in the megasporangium. The abortion

of all thirty-two spores in the megasporangium, which holds the central position in the microsporocarp, could be considered a further step in this reduction.

What causes the abortion in one case of thirty, in another of thirty-one, and in a third of thirty-two megaspores, is a question not easily determined. It would seem largely a matter of chance, since there is no external factor or group of factors responsible. Very often megasporocarp and microsporocarp lie side by side, having begun their development from two apical cells which were formed by the division of a single cell, and having continued growth with their surroundings as nearly alike as it is possible to make them. On the other hand, there is no internal condition that we can say, definitely, must be responsible for this. There is of course the possibility that the whole thing is a question of food supply, as STRASBURGER suggested. If there should be some change in the cells of the stalk below the megasporangium making them impermeable to any of the materials which are necessary for growth in the sporangium, the inhibition of growth there would be a natural result. That the food supply thus kept from the megasporangium should then be used for the further development of the microsporangium at the base, seems a plausible supposition. In cases where the cells of the stalk later grow up into the megasporangial cavity, one would have to suppose a regaining of permeability of the cells to food materials. Such changes in the cells from permeability to a given substance to impermeability and back to permeability are quite possible physiologically.

SUMMARY

1. The early development of megasporocarp and microsporocarp are exactly similar. Each begins development with a single megasporangium and a sporocarp wall. When the megasporangium has reached the stage in which eight spore mother cells are in synapsis, some of the outer cells of the stalk enlarge and become the apical cells of young microsporangia. Growth in the microsporangia and megasporangium now goes on simultaneously for some time. The tapetal walls of the megasporangium disappear and the mother cells separate from each other and round off, while the microsporangia at the base continue development, differing in appearance from the

megasporangium only in having long slender stalks and later forming sixteen rather than eight spore mother cells. Before the primary wall cell is cut off in the oldest of the microsporangia, the megaspore mother cells have divided simultaneously to form eight tetrads. Up to this point the development is the same, whether the structure is to be a megasporocarp or a microsporocarp.

2. Further development of the megasporocarp shows one of the thirty-two megaspores continuing growth, while the other thirty-one abort, and the microsporangia at the base of the megasporangium cease growth.

3. Further development of the microsporocarp shows all thirty-two megaspores aborting, while the young microsporangia increase rapidly in size and number. The megasporangium usually collapses so that it is not readily seen in the mature microsporocarp.

This investigation was carried on under the direction of Professors John M. Coulter and Charles J. Chamberlain, The University of Chicago.

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EXPLANATION OF PLATES XXXI AND XXXII

The figures were all made with a Zeiss camera lucida and in all cases, except *fig. 11a*, a Zeiss ocular 4 and Zeiss 2^{mm} objective were used, giving a magnification of 430 diameters after a ½ reduction is made in the reproduction.

Abbreviations: *a*, Anabaena; *am*, abortive megaspore; *as*, abortive megasporangium; *at*, abortive megaspore tetrad; *m*, functional megaspore; *mc*, microsporangia; *mcm*, microspore mother cells; *mm*, megaspore mother cell; *mt*, megaspore tetrads; *s*, sporocarp wall; *t*, tapetum.

PLATE XXXI

Figs. 1–8 give the stages in the development in which the megasporocarp and microsporocarp are similar.

FIG. 1. A very young sporocarp showing the beginning of the megasporangium and sporocarp wall.

FIG. 2. A stage later than *fig. 1.*

FIG. 3. A still later stage; the primary tapetal cells have been cut off in the megasporangium.

FIG. 4. A considerably later stage; the megasporangium is in the mother-cell stage, but no microsporangial primordia have yet appeared.

FIG. 5. A little later than *fig. 4;* the microsporangial primordia are appearing.

FIG. 6. The microsporangia have begun growth; in the megasporangium the tapetal walls have disappeared.

FIG. 7. The heterotypic division of the megaspore mother cells; growth in the microsporangium continuing.

PLATE XXXII

FIG. 8. A still later stage.

FIG. 9. A megasporocarp that can be readily recognized as such; microsporangia have ceased growth.

Figs. 10–13 show conditions found in the microsporocarp.

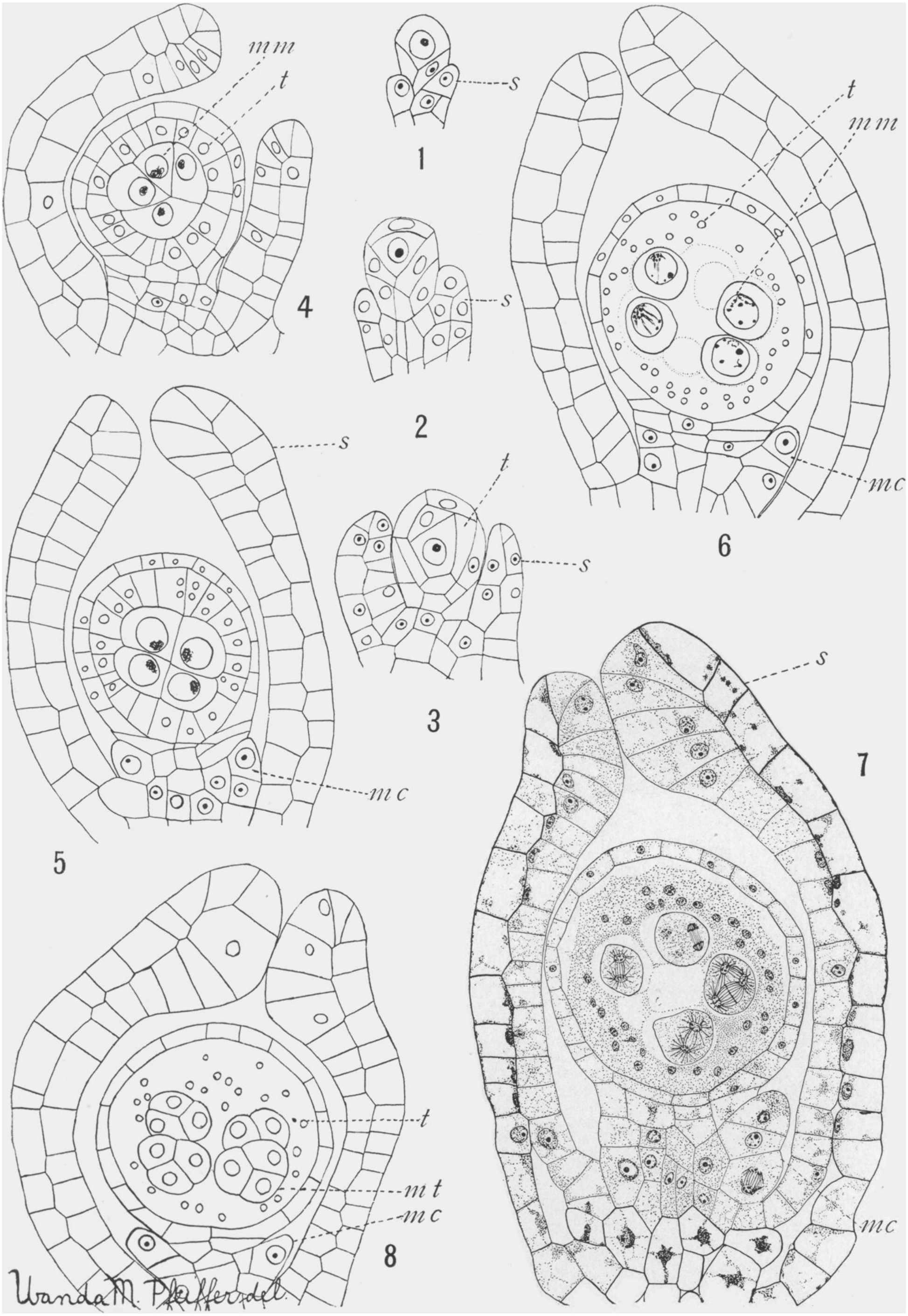
FIG. 10. A microsporocarp of about the same age as the sporocarp in *fig. 9;* the microsporangia are appearing in large numbers.

FIG. 11. The central portion of an older microsporocarp, showing the microsporangium with microspore mother cells, *mcm.*

FIG. 11*a*. An outline sketch of the whole sporocarp, a portion of which is enlarged in *fig. 11.*

FIG. 12. One of the abortive megasporangia, into which the cells of the stalk are beginning to grow.

FIG. 13. An abortive megasporangium which has been nearly filled by the upward growth of the cells of the stalk.



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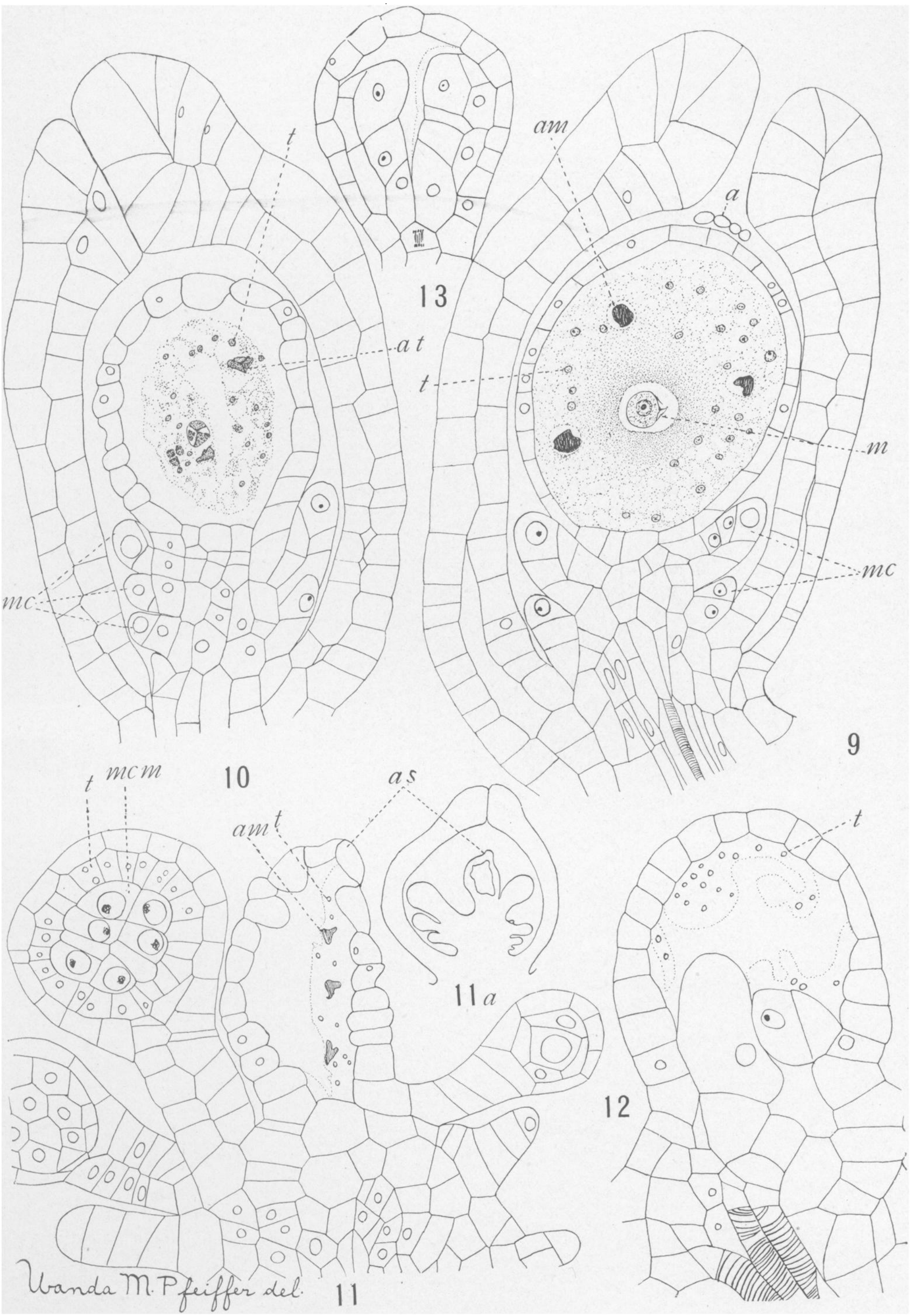

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